ALLOZYME DIVERSITY IN *ELEUTHEROCOCCUS SENTICOSUS* AND *E. BRACHYPUS* (ARALIACEAE) FROM CHINA AND ITS IMPLICATION FOR CONSERVATION

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ABSTRACT

The widespread *Eleutherococcus senticosus* (Rupr. et Maxim.) Maxim. is threatened because of over-harvesting of its root bark for medicinal uses. The geographically restricted *E. brachypus* becomes endangered due to habitat loss in the Loess Plateau of China. To facilitate the development of conservation strategies, genetic diversity of both species was measured at 26 isozyme loci (12 enzymes). *Eleutherococcus brachypus* had a low proportion of polymorphic loci ($P_{.5}$=19.2%; P_p=13.1%) and low genetic diversity ($H_{.5}$=0.063; H_{ep}=0.031). The genetic variability of the widespread *E. senticosus* was higher ($P_{.5}$=26.9%; P_p=20.76%; $H_{.5}$=0.094; H_{ep}=0.059) than that of the restricted *E. brachypus*. Population differentiation of *E. brachypus* (G_{st}=0.531) was greater than that of *E. senticosus* (G_{st}=0.383). Cluster analysis showed that populations of *E. senticosus* in Northeast or North China are genetically closer within each region than between the two regions. Populations of *E. senticosus* in Northeast China have a higher level of genetic diversity, and these populations need to be conserved with greater priority. Populations of *E. brachypus* in the Loess Plateau have been highly fragmented, and all populations of the species should be protected to maximize its genetic diversity.

CHINESE ABSTRACT

刺五加（*Eleutherococcus senticosus* (Rupr. et Maxim.) Maxim.）分布于华北和东北地区，由于人类的过度利用而使该物种受到威胁；短柄五加由于分布于黄土高原这一植被非常稀少的特殊地理地带同样有绝灭的危险。为了制定这些物种的有效保护措施，我们对这两个物种的遗传多样性进行了分析。我们每个物种各采集了5个居群的样品，共197份；分析了12个酶系统，共得到26位点。数据分析结果表明，短柄五加物种水平的多态位点为19.2%，居群水平的多态位点平均为13.1%；物种水平的期望杂合度为0.063，居群水平的期望杂合度平均为0.031。刺五加物种水平的多态位点为26.9%，居群水平的多态位点平均为20.76%；物种水平的期望杂合度为0.094，居群水平的期望杂合度平均为0.059。短柄五加居群间的分化系数（G_{st}=0.531）高于刺五加（G_{st}=0.383）。聚类分析结果显示，东北地区和华

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北地区的刺五加地区内部的居群之间具有更高的遗传相似性。东北地区的刺五加居群具有比华北地区的居群更高的等位酶多样性，从而更具有保护价值。短柄五加的遗传多样性没有明显的地理规律。考虑到黄土高原非常稀少的植被，短柄五加在水土保持中的重要作用，加上其居群数目少，遗传多样性低，建议保护所有自然群体以维持该物种的遗传多样性。

INTRODUCTION

Understanding of the levels of genetic diversity is important in designing conservation strategies for rare and endangered species (Holsinger & Gottlieb 1991; Hamrick & Godt 1996; Rieseberg & Swensen 1996; Newton et al. 1999; Gitzendanner & Soltis 2001). It is estimated that approximately 3000–4000 species (15%) of the vascular plants in China are endangered or threatened (Fu & Jin 1992). Since the 1970's, major efforts have been made to establish nature reserves to protect rare and endangered species and their communities. At present, few studies have been conducted to examine the genetic diversity of endangered species in China. Genetic data are needed from model plant groups in China to help design conservation efforts when resources are limited.

Eleutherococcus senticosus [= *Acanthopanax senticosus* (Rupr. et Maxim.) Harms] (Araliaceae) distributed in China, North Korea and Far East of Russia, is commonly known as Siberian ginseng (Soejarto & Farnsworth 1978; Farnsworth et al. 1985; Duke 1989) and is considered to be of high medicinal value. It has been used for hundreds of years in China as a tonic (Hu 1980). Studies have been conducted (e.g., Xu et al. 1983; Zhao et al. 1990, 1991, 1993) to analyze its chemical constituents. Polysaccharides extracted from its leaves and roots have been reported to inhibit tumor cell proliferation (Xie et al. 1989; Liang et al. 1994) and to have antiviral activity (Glatthaar-Saalmüller et al. 2001). Glucosides (such as liriodendrin) extracted from root or stem bark have also been reported to have an effect similar to those of that of ginseng (*Panax* spp.) (Slacanin et al. 1991), or as an adaptogen that exerts effects on both sick and healthy people by "correcting" any dysfunctions with no or few side effects (Davydov & Krikorian 2000). Industries have been developed to manufacture herbal products using *E. senticosus*. Herbal and pharmaceutical producers have been using material of the species collected from natural habitats, which has led to rapid destruction of natural populations. Although the species has a wide distribution in China, ranging from the North (Shanxi and Hebei provinces) to the Northeast (Liaoning, Jilin and Heilongjiang provinces) (Fig. 1), it is now listed in the "China Plant Red Data Book" as a vulnerable species (Fu & Jin 1992) for its economical importance.

Eleutherococcus brachypus (Harms) Nakai is a rare endemic species restricted to the Loess Plateau (southeastern Gansu and central Shaanxi provinces) of Northwest China (Fig. 1), which is heavily populated and has highly fragmented vegetation. *Eleutherococcus brachypus* is a clonal species with small populations (Wang et al., 1997), but has suffered from habitat loss, and is thus rare. Seeds of *E. brachypus* are usually not well developed, requiring 1.5 years of

after-ripening (Tian et al. 1998). Wang et al. (1997) reported that insect visitation was necessary for seed set. Yan et al. (1997) investigated genetic diversity of this species using three populations from Yanán City, Shaanxi Province, and reported that the percentages of polymorphic RAPD bands were relatively low, 5.4%, 18.5%, and 27.7%, respectively.

Both *Eleutherococcus senticosus* and *E. brachypus* are shrubs with similar ecological preferences of sunny habitats, but different in geographic ranges. *Eleutherococcus senticosus* has a wide distribution, whereas *E. brachypus* is a rare endemic confined to the Loess Plateau of Northwest China (Fig. 1). This paper assesses the genetic diversity of these two congeneric species with allozyme markers using starch gel electrophoresis and discusses the implications for conservation.

MATERIALS AND METHODS

Sampling

Five populations of *E. senticosus* from five provinces in China and five populations of *E. brachypus* from North Shaanxi to South Gansu were sampled, covering nearly the full range of distribution of both species (Fig. 1, Table 1). Three populations of three other congeners: *E. giraldii* (Harms) Nakai, *E. gracilistylus* (W.W. Smith) S.Y. Hu, and *E. sessiliflorus* (Rupr. et Maxim.) S.Y. Hu (Table 1) were included in the UPGMA cluster analysis as comparisons.

One to two year old twigs were collected in the spring from about 20 individuals in each population. Populations of *E. brachypus* were small and the boundaries were easily determined. Samples were collected throughout the populations. Populations of *E. senticosus* were usually large with hundreds of individuals, and samples were collected at an interval of at lest 50 meters to minimize the possibility of collecting two samples from a single clone. The twigs were then kept in sealed moist plastic bags.

Electrophoresis

In the laboratory, the cut surface of each twig was immersed in water and incubated in a humid environment. When the first leaf appeared, the bud was removed and ground on ice with grinding buffer after removing bud scales. The grinding buffer (Tris-malate grinding buffer-PVP solution) was prepared following Soltis et al. (1983) with the substitution of sodium bisulfite for sodium metabisulfite. DMSO was added to the mixture to a final concentration of 10% before adjusting pH to 7.5. The enzyme solution was absorbed onto wicks, which were frozen (-80°C) until electrophoresis. Electrophoresis was carried out on horizontal starch (Sigma cat no. S4501) gels at 4°C. Four buffer systems (electrode buffer / gel buffer) were used to assay12 enzymes (Wang 1998): (1) 0.4M Citric acid trisodium salt (pH adjusted to 7.0 using 1.0M HCl) / 0.02M Histidine•HCl (pH adjusted to 7.0 using 1.0M NaOH) for aconitate hydratase

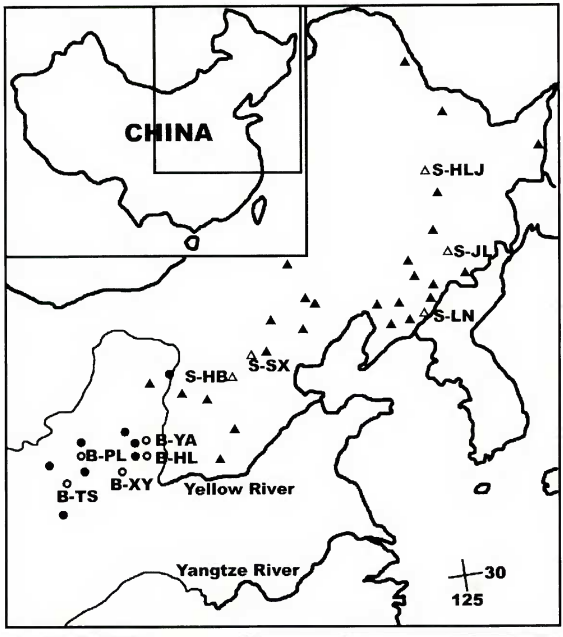

Fig. 1. Distribution of *Eleutherococcus brachypus* (●) and *E. senticosus* (▲) in China. Open triangles and circles indicate populations sampled.

(ACO, E. C. 4.2.1.3), fructose-bisphosphate aldolase (FBA, E. C. 4.1.2.13) and glyceraldehydes-3-phosphate dehydrogenase (G3PD, E. C. 1.2.1.12); (2) 0.3M boric acid (pH adjusted to 8.6 using NaOH) / 0.015M Tris (pH adjusted to 7.8 using citric acid) for aminopeptidase (AMP, E. C. 3.4.11.1), hexokinase (HEX, E. C. 2.7.1.1), phosphoglucomutase (PGM, E. C. 5.4.2.2) and triose-phosphate isomerase (TPI, E. C. 5.3.1.1); (3) 0.374M boric acid (pH adjusted to 8.0 using LiOH) / 0.033M Tris+0.005M citric acid+0.004M LiOH +0.030M boric acid for aspartate aminotransferase (AAT, E. C. 2.6.1.1), alcohol dehydrogenase (ADH, E. C. 1.1.1.1) and

TABLE 1. Population localities, symbols and voucher specimens of *Eleutherococcus brachypus*, *E. senticosus* and close congeners.

Locality	Symbol	Voucher (PE)
Eleutherococcus senticosus (Rupr. et Maxim.) Maxim.		
Mao'ershan, Shangzhi County, Heilongjiang Province	S-HLJ	*Zhou 009*
Changbaishan Nature Reserve of CAS, Antu County, Jilin Province	S-JL	*Zhou 010*
Baishiiazi Nature Reserve, Kuandian County, Liaoning Province	S-LN	*Zhou 011*
Mount Wutaishan, Shanxi Province	S-SX	*Zhou 013*
East Lingshan, Hebei	S-HB	*Zhou 014*
Eleutherococcus brachypus (Harms) Nakai		
Nanniwang, Yan'an City, Shaanxi Province	B-YA	*Zhu 950004*
Nanshan, Huanglong County, Shaanxi Province	B-HL	*Zhou 007*
Yangjiadian, Xunyi County, Shaanxi Province	B-XY	*Zhou 003*
Mount Kongtongshan, Pingliang City, Gansu Province	B-PL	*Zhu 95016*
Caijiashan, Lu'ergou, Tianshui City, Gansu Province	B-TS	*Zhou 002*
Eleutherococcus gracilistylus (W.W.Sm.) S.Y.Hu		
Angmenkou, Kanxian County, Gansu Province		*Zhou 006*
Eleutherococcus giraldii (Harms) Nakai		
Mount Lianhuashan, Kanle County, Gansu Province		*Zhou 005*
Eleutherococcus sessiliflorus (Rupr. et Maxim.) S.Y.Hu		
Baishilazi Nature Reserve, Kuandian County, Liaoning Province		*Zhou 012*

NADH-diaphorase (DIA, E. C. 1.6.2.2); and (4) 0.04M citric acid [pH adjusted to 7.5 using N-(3-aminopropyl)-morpholine] / 1:19 dilution of electrode buffer for isocitrate dehydrogenase (IDH, E. C. 1.1.1.42) and shikimate dehydrogenase (SKD, E. C. 1.1.1.25). Enzymes were visualized using stains in agar overlays except for AAT and AMP which were stained in buffer solutions.

Data analysis

Stained gels were photographed and the banding patterns were then drawn. The alleles at each locus were designated with letters a, b, and c, from the longest migration distance to the shortest. The resulting genetic data (genotypes) were analyzed with Biosys-1 (Swofford & Selander 1989) for each species. For each population, the allele frequencies, mean number of alleles per locus, percentage of polymorphic loci, heterozygosity observed and expected under Hardy-Weinberg equilibrium, F-statistics and unbiased genetic similarities/distances (Nei 1978) were computed. All the populations were analyzed to generate a dendrogram using UPGMA.

RESULTS

Twenty-six putative loci from 12 enzyme systems were interpretable on the basis of simple Mendelian genetics (Table 2). Nineteen loci exhibited polymorphisms in one or both species. The frequency of one allele often dominated over the others in a given population (Table 2).

Genetic diversity of *E. senticosus*

There were 13 polymorpic loci in *E. senticosus* (Table 2). The two regions of activity for AAT were designated as *Aat-1* and *Aat-2*. *Aat-1* had 2 alleles, the rare one *Aat-1b* and the common one *Aat-1c*. *Aat-2* had 3 alleles, *Aat-2a*, *Aat-2b*, and *Aat-2c* with *Aat-2b* being the common one. Only one locus was detected for ADH with 2 alleles, *Adh-a* and *Adh-b*, which were present in heterozygosity with low frequencies. Both *Amp-1* and *Amp-2* had 2 alleles, with the allele frequencies varying among populations. *Dia-1* exhibited polymorphism only in the S-JL population. Two alleles were detected on *G3pd-2* and *Hex-1*, respectively. The rare alleles on *Idh-2*, *Skd-1*, *Skd-2*, *Skd-3*, *Tpi-1*, and *Tpi-2* were unique to this species. Locus duplications were observed on PGM and TPI.

Eleutherococcus senticosus maintained a higher level of genetic diversity. The percentages of polymorphic loci ranged from 11.5% to 30.8% with a mean of 26.9% (Table 3). The mean number of alleles averaged over populations was 1.26, and the expected heterozygosity under Hardy-Weinberg equilibrium averaged over populations was 0.059. The population S-JL from Jilin Province (A=1.3; P=30.8%; H_e=0.073) and the population S-LN from Liaoning Province (A=1.3; P=23.1%; H_e=0.077) exhibited the highest genetic diversity. The southwest peripheral population S-SX from Shanxi Province had the lowest (A=1.2; P=11.5%; H_e=0.025).

At the species level, the mean number of alleles per locus was 1.7, and 26.9% of the loci were polymorphic. The expected heterozygosity was 0.094. The genetic diversity was maintained at 13 polymorphic loci, especially at *Amp-1*, *Amp-2*, *G3pd-2*, *Idh-2*, *Skd-3* and *Tpi-1* (Ht>0.2, Table 4). Among these loci only *Amp-1* and *Idh-2* contributed more to interpopulational than to intrapopulational variation (G_{St}>0.5). Of the mean total genetic diversity (H_t=0.094), only 38.3% was maintained within populations (H_s=0.058, D_{St}=0.036).

Genetic diversity of *E. brachypus*

There were 10 polymorphic loci detected in five populations of this species (Table 2). *Aat-1a* was unique to B-XY population. The alleles of *Aat-2a* and *Aat-2c* were infrequent. *Aco-1a* was common in B-XY population but rare or absent in the other populations. The unique allele *Adh-c* was present in heterozygosity with low frequencies. *Dia-2b* and *Dia-2c* were characteristic of this species, which exhibited intrapopulational variation. Nearly complete divergence of alternative allele fixation was found at the three loci of IDH between *E. brachypus* and *E. senticosus*. Locus duplication was not observed at PGM. Instead, both loci *Pgm-1* and *Pgm-2* were polymorphic.

The genetic diversity of *E. brachypus* at the populational level was relatively low compared to that of *E. senticosus* (Table 3). The percentage of polymorphic loci varied from 3.8% to 23.1% with a mean of 13.1%. The mean number of alleles

TABLE 2. Allele frequencies in 10 populations of *Eleutherococcus brachypus* and *E. senticosus* (N indicates sample size).

		Populations									
Locus	Allele	Eleutherococcus brachypus					Eleutherococcus senticosus				
		B-TS	B-PL	B-XY	B-YA	B-HL	S-HLJ	S-JL	S-LN	S-SX	S-BJ
	(N)	20	19	15	14	20	19	20	20	20	20
Aat-1	*a*	–	–	0.033	–	–	–	–	–	–	–
	b	–	–	–	–	–	–	0.200	0.175	–	–
	c	1.000	1.000	0.967	1.000	1.000	1.000	0.800	0.825	1.000	1.000
Aat-2	*a*	–	–	–	0.036	–	0.132	–	–	–	–
	b	0.950	1.000	1.000	0.964	0.900	0.868	0.975	1.000	1.000	1.000
	c	0.050	–	–	–	0.100	–	0.025	–	–	–
Aco-1	*a*	–	–	1.000	0.071	–	–	–	–	–	–
	b	1.000	1.000	–	0.929	1.000	1.000	1.000	1.000	1.000	1.000
Aco-2	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Adh	*a*	0.050	0.026	–	0.071	0.025	–	0.100	–	–	0.075
	b	0.950	0.947	1.000	0.857	0.975	1.000	0.900	1.000	1.000	0.925
	c	–	0.026	–	0.071	–	–	–	–	–	–
Amp-1	*a*	–	0.026	–	–	–	1.000	0.750	0.275	0.150	0.975
	b	1.000	0.974	1.000	1.000	1.000	–	0.250	0.725	0.850	0.025
Amp-2	*a*	–	–	–	–	–	0.447	0.300	–	0.025	–
	b	1.000	1.000	1.000	1.000	1.000	0.553	0.700	1.000	0.975	1.000
Dia-1	*a*	1.000	1.000	1.000	1.000	1.000	1.000	0.925	1.000	1.000	1.000
	b	–	–	–	–	–	–	0.075	–	–	–
Dia-2	*a*	0.850	0.789	0.933	0.286	1.000	1.000	1.000	1.000	1.000	1.000
	b	0.075	0.105	0.033	0.357	–	–	–	–	–	–
	c	0.075	0.105	0.033	0.357	–	–	–	–	–	–
Fba	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
G3pd-1	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
G3pd-2	*a*	0.150	1.000	1.000	–	0.050	–	–	0.300	–	0.350
	b	0.850	–	–	1.000	0.950	1.000	1.000	0.700	1.000	0.650
Hex-1	*a*	1.000	1.000	1.000	1.000	0.950	1.000	0.950	0.950	0.975	1.000
	b	–	–	–	–	0.050	–	0.050	0.050	0.025	–
Hex-2	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Idh-1	*a*	–	–	–	–	–	1.000	1.000	1.000	1.000	1.000
	b	1.000	1.000	1.000	1.000	1.000	–	–	–	–	–
Idh-2	*a*	–	–	–	–	–	1.000	1.000	1.000	0.050	0.175
	b	1.000	1.000	1.000	1.000	1.000	–	–	–	0.950	0.800
	c	–	–	–	–	–	–	–	–	–	0.025
Idh-3	*a*	–	–	–	–	–	1.000	1.000	1.000	1.000	1.000
	b	1.000	1.000	1.000	1.000	1.000	–	–	–	–	–
Pgm-1	*a*	0.300	–	–	–	0.100	–	–	–	–	–
	b	0.700	0.947	1.000	1.000	0.750	1.000	1.000	1.000	1.000	1.000
	c	–	0.053	–	–	0.150	–	–	–	–	–
Pgm-2	*a*	0.950	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	b	0.050	–	–	–	–	–	–	–	–	–

TABLE 2. continued

Locus	Allele	Populations									
		Eleutherococcus brachypus					Eleutherococcus senticosus				
Skd-1	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Skd-2	*a*	–	–	–	–	–	0.053	–	–	–	–
	b	1.000	1.000	1.000	1.000	1.000	0.947	1.000	1.000	1.000	1.000
Skd-3	*a*	–	–	–	–	–	0.132	–	–	0.025	0.500
	b	1.000	1.000	1.000	1.000	1.000	0.868	1.000	1.000	0.900	0.500
	c	–	–	–	–	–	–	–	–	0.075	–
Tpi-1	*a*	–	–	–	–	–	–	–	0.300	–	0.025
	b	1.000	1.000	1.000	1.000	1.000	0.684	0.950	0.400	1.000	0.950
	c	–	–	–	–	–	0.316	0.050	0.300	–	0.025
Tpi-2	*a*	1.000	1.000	1.000	1.000	1.000	1.000	0.900	0.950	1.000	1.000
	b	–	–	–	–	–	–	0.100	0.050	–	–
Tpi-3	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Tpi-4	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

TABLE 3. Genetic variability at 26 loci in the populations of *Eleutherococcus brachypus* and *E. senticosus* (standard errors in parentheses). N indicates the sample size per locus; *A* indicates the mean number of alleles per locus; *P* indicates the percentage of polymorphic loci*; *Ho* indicates the observed heterozygosity; and *He* indicates the expected heterozygosity under Hardy-Weiberg equilibrium.**

Population	N	A	P	H_o	H_e
		Eleutherococcus brachypus			
B-TS	20	1.3(0.1)	23.1	0.013(0.008)	0.048(0.021)
B-PL	19	1.2(0.1)	11.5	0.014(0.009)	0.024(0.015)
B-XY	15	1.1(0.1)	3.8	0.005(0.004)	0.008(0.006)
B-YA	14	1.2(0.1)	11.5	0.047(0.029)	0.045(0.028)
B-HL	20	1.2(0.1)	15.4	0.006(0.004)	0.032(0.018)
Mean		1.2	13.06	0.017	0.031
Species level	88	1.5(0.1)	19.2	0.016(0.009)	0.063(0.026)
		Eleutherococcus senticosus			
S-HLJ	19	1.2(0.1)	19.2	0.030(0.017)	0.059(0.027)
S-JL	20	1.3(0.1)	30.8	0.012(0.007)	0.073(0.025)
S-LN	20	1.3(0.1)	23.1	0.042(0.027)	0.077(0.034)
S-SX	20	1.2(0.1)	11.5	0.019(0.011)	0.025(0.013)
S-BJ	20	1.3(0.1)	19.2	0.012(0.006)	0.062(0.028)
Mean		1.3	20.8	0.023	0.059
Species level	99	1.7(0.1)	26.9	0.023(0.007)	0.094(0.029)

Note. * A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95

** Unbiased estimate (see Nei 1978)

TABLE 4. Genetic diversity across populations of *E. brachypus* and *E. senticosus*. H_t indicates the total gene diversity; H_s indicates gene diversity within populations; D_{st} indicates the gene diversity between populations; and G_{st} is the ratio of D_{st}/H_t.

Locus	H_t	H_s	D_{st}	G_{st}
		Eleutherococcus brachypus		
Aat-1	0.013	0.013	0.000	0.000
Aat-2	0.072	0.069	0.003	0.042
Aco-1	0.337	0.027	0.310	0.920
Adh	0.105	0.101	0.004	0.038
Amp-1	0.010	0.010	0.000	0.000
Dia-2	0.378	0.282	0.096	0.254
G3pd-2	0.493	0.070	0.423	0.858
Hex-1	0.020	0.019	0.001	0.050
Pgm-1	0.218	0.185	0.033	0.151
Pgm-2	0.020	0.019	0.001	0.050
Mean	0.064	0.030	0.034	0.531
		Eleutherococcus senticosus		
Aat-1	0.139	0.122	0.017	0.122
Aat-2	0.061	0.055	0.006	0.098
Adh	0.068	0.064	0.004	0.059
Amp-1	0.466	0.215	0.251	0.539
Amp-2	0.261	0.192	0.069	0.264
Dia-1	0.030	0.028	0.002	0.067
G3pd-2	0.226	0.175	0.051	0.226
Hex-1	0.049	0.048	0.001	0.020
Idh-2	0.461	0.085	0.376	0.816
Skd-2	0.021	0.020	0.001	0.048
Skd-3	0.254	0.182	0.072	0.283
Tpi-1	0.342	0.257	0.085	0.249
Tpi-2	0.058	0.055	0.003	0.052
Mean	0.094	0.058	0.036	0.383

averaged over populations was 1.2, and the expected heterozygosity under Hardy-Weinberg equilibrium averaged over populations was 0.031. The population B-TS from Tianshui, Gansu Province, showed the highest genetic diversity (A=1.3; P=23.1%; H_e=0.048), while the population B-XY from Xunyi, Shaanxi Province, showed the lowest diversity (A=1.1; P=3.8%; H_e=0.008).

Eleutherococcus brachypus exhibited an average of 1.5 alleles per locus, and 19.2% of loci were polymorphic (Tables 3 & 4). The expected heterozygosity under Hardy-Weinberg equilibrium was 0.063. The genetic diversity was maintained at 10 polymorphic loci, notably at *Aco-1*, *Dia-2*, *G3pd-2* and *Pgm-1*. The mean total gene diversity (H_t) was 0.064. Nearly half of the total genetic diversity occurred within populations (H_s=0.030). Among the loci with high H_t values, *Dia-2* and *Pgm-1* showed most genetic variation within populations (G_{st}

was 0.254 and 0.151, respectively), while *Aco-1*(G_{st}=0.858) and *G3pd-2* (G_{st}=0.920) showed genetic variation primarily among populations.

Relationships among populations

Genetic identity between populations within each species was higher than 0.9, and the genetic distances were lower than 0.09 (Table 5). The average distances between the populations were 0.043 (±0.028) and 0.047 (±0.019) for *E. brachypus* and *E. senticosus*, respectively. The dendrogram generated from genetic identity data using UPGMA (Fig. 2) showed that populations of *E. senticosus* from Northeast China were more similar to one another than with those from North China. The population B-XY of *E. brachypus* differentiated most significantly from other populations of the species.

DISCUSSION

Overall genetic diversity within species

The levels of allozyme variation in *E. senticosus* and *E. brachypus* were relatively low at both species and populational levels in comparison with those of other species with similar attributes (Hamrick & Godt 1990). For example, P_s (26.9%) and H_{es} (0.094) of *E. senticosus* were about half the averages (64.7% and 0.177, respectively) of woody plants at the species level. Hamrick & Godt (1996) reported an average within species heterozygosity (H_{es}) for widespread and endemic species of 0.202 and 0.096, respectively. The H_{es} for the widespread *E. senticosus* was 0.094 and that of the restricted endemic species *E. brachypus* was 0.063. The allozyme heterozygosity within each of the two *Eleutherococcus* is thus considerably lower than the averages reported in the literature.

The percentages of polymorphic allozyme loci reported in this study correspond well to DNA-RAPD polymorphism in *E. brachypus* (3.8–23.1% vs 5.4–27.2%, Yan et al. 1997), but differ sharply from those of *E. senticosus* (11.5–30.8% vs 91.3–97.6%, Dai et al. 1998). However, the report of highly polymorphic RAPD bands in *E. senticosus* likely represents an overestimate because amplification failure from some individuals was interpreted as a lack of bands by Dai et al. (1998). Our allozyme data seem to be more reliable in presenting the overall genetic diversity of the species investigated.

Levels of genetic diversity between species

There is significant disparity between *E. brachypus* and *E. senticosus* in overall genetic diversity (Tables 3 & 4), with *E. senticosus* maintaining a higher level of genetic diversity than *E. brachypus*. These two species appears to be closely related, but not sister taxa. They differ in several characters that may influence genetic diversity. First, they have highly different distributional ranges. *Eleutherococcus senticosus* is widespread across several thousand kilometers, from North to Northeast China and adjacent countries (North Korea and Far East of Russia). By contrast, *E. brachypus* is restricted to the Loess Plateau of

TABLE 5. Matrix of Nei's (1978) unbiased genetic identity (below diagonal) and distance (above diagonal) between populations of *E. brachypus* and *E. senticosus*.

	B-TS	B-PL	B-XY	B-YA	B-HL	S-HLJ	S-JL	S-LN	S-SX	S-BJ
B-TS		0.032	0.074	0.013	0.002	0.199	0.169	0.154	0.090	0.147
B-PL	0.969		0.040	0.048	0.039	0.239	0.210	0.170	0.128	0.157
B-XY	0.929	0.961		0.089	0.080	0.289	0.258	0.216	0.171	0.204
B-YA	0.987	0.953	0.914		0.017	0.211	0.181	0.170	0.101	0.163
B-HL	0.998	0.962	0.923	0.983		0.193	0.164	0.152	0.086	0.146
S-HLJ	0.820	0.788	0.749	0.810	0.825		0.009	0.039	0.079	0.050
S-JL	0.844	0.811	0.772	0.834	0.848	0.991		0.025	0.057	0.050
S-LN	0.857	0.844	0.806	0.843	0.859	0.962	0.975		0.053	0.070
S-SX	0.914	0.880	0.843	0.904	0.918	0.924	0.944	0.948		0.041
S-BJ	0.863	0.855	0.815	0.849	0.864	0.952	0.951	0.933	0.960	

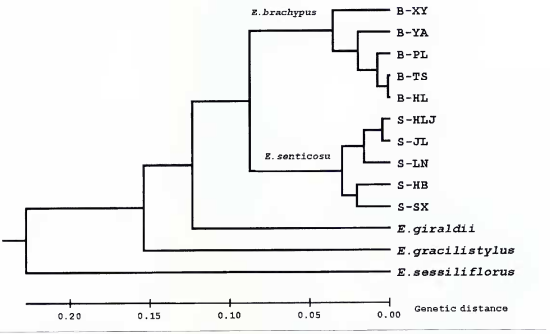

FIG. 2. Cluster analysis (UPGMA) of populations of *Eleutherococcus* based on Nei's unbiased genetic distance.

southeastern Gansu Province and central Shaanxi Province. Another major difference is the breeding system. *Eleutherococcus senticosus* is reported to be trioecious and protandrous (Liu et al. 1997a, 1998). But because individuals with hermaphroditic flowers are very rare (Liu et al. 1997b), this species is functionally dioecious with insect-mediated outcrossing (Liu et al. 1998a). *Eleutherococcus brachypus* has hermaphrodite flowers. Both selfing and insect-mediated outcrossing are important in its sexual reproduction. Similar to *E. senticosus*, anthers of *E. brachypus* start to shed pollen at least five days before

the receptivity of stigmas of the same flower (Wang et al. 1997), suggesting outcrossing. Wang et al. (1997) proposed that "outcrossing" within local populations may represent selfing in a broad sense because local populations may be ramets of a single clone. But our study has shown that there is genetic variation within local populations. Thus the interpretation of a local population to be a group of ramets from a single clone is not supported (also see Yan et al. 1997).

The degree of human impact may also explain the difference in genetic diversity between these two species. The populations of *E. senticosus* assayed in this study have not suffered serious disturbance because they were mostly in nature reserves. In contrast, all populations of *E. brachypus* sampled were seriously disturbed, and they were finely fragmented due to land reclamation for farming.

Genetic diversity among populations

Considerable genetic variation was detected among populations (Table 3). The level of genetic diversity varies across localities in *E. senticosus*. The population S-LN in Liaoning Province is the center of the present distribution of this species, and the genetic diversity (*He*) is highest. The population S-SX in Shanxi Province is peripheral and its genetic diversity is the lowest. This pattern has also been reported in many other plant species (see Crawford 1990; Hamrick & Godt 1996).

In *E. brachypus*, however, the Tianshui population (B-TS) near the westernmost range of the species has the highest genetic diversity, whereas the Xunyi population (B-XY) near the distributional center shows the lowest level of genetic diversity. This unusual pattern may be due to a greater impact of human disturbance on *E. brachypus*.

Implications for conservation

In *Eleutherococcus senticosus* in China, populations near the distributional center have a higher level of genetic variation. From this center to the periphery, polymorphism decreases. The low genetic diversity in the populations of North China is expected due to their peripheral positions. When the entire distributional range (including the Far East of Russia) is considered, the population S-HLJ is also central. The relatively low genetic diversity of this population suggests that it may have suffered from genetic loss. Population destruction of *E. senticosus* has been serious in Heilongjiang Province.

Although the genetic structure of *E. senticosus* has not been seriously damaged, overharvesting should be prevented to maintain the sustainability of this species. Recent establishment of nature reserves in Northeast China (the center of genetic variation of this species in China) has been successful in protecting some populations of *E. senticosus*. Demographic investigation outside the reserves every few years is needed to determine the reduction rate of populations.

If it is rapid, use of this species should be controlled, and *ex situ* conservation of special genetic resources may be adopted in nearby nature reserves.

Conservation of *E. brachypus* is urgent because considerable genetic loss has occurred. No significant correlation has been found between genetic differentiation of populations and their geographical distances from the distributional center, suggesting that genetic structure has been altered. The vegetation of the Loess Plateau has been seriously fragmented. In order to maximize the conservation of genetic diversity, all natural populations should be protected. If nature reserves are to be established to conserve populations of this important species, priorities should be given to those in southern Gansu Province, where genetic diversity is high and habitat loss is serious.

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